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# Sampling for Organic Chemicals and Microorganisms in the Subsurface

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Procedures currently utilized by the Ground Water Research Branch of the Environmental Protection Agency for sampling for organic pollutants and microorganisms in ground waters and subsurface earth solids are presented. Technology is described for construction of wells capable of providing representative, uncontaminated samples of ground water in compact alluvial formations at relatively shallow depths and for obtaining cores of subsurface earth solids suitable for organic and microbial analyses in similar circumstances. Methods for acquisition of grab samples of ground water suitable for total organic and microbial analyses and for analyses of volatile organics are presented. Continuous sampling of organics in ground waters lying within approximately 7.5 m (25 ft) of the surface by sampling units utilizing selected absorbents is described, including details of adsorbent columns, configuration of and housings for sampling systems, and sample handling. Procedures for handling and processing of core materials to produce samples amenable to analytical methods for organics and microorganisms are also presented.

The procedures described provide a basic capability for sampling for organic pollutants and microorganisms in relatively shallow subsurface environments, and have potential application in many investigations pertaining to ground-water pollution. Additional research is needed, however, to further evaluate, improve, and extend their capabilities. This report covers a period from July 1975 to January 1977, and work was completed as of May 1977.

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# SAMPLING FOR ORGANIC CHEMICALS AND MICROORGANISMS IN THE SUBSURFACE

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## FOREWORD

The Environmental Protection Agency was established to coordinate administration of the major Federal programs designed to protect the quality of our environment.

An important part of the Agency's effort involves the search for information about environmental problems, management techniques, and new technologies through which optimum use of the Nation's land and water resources can be assured and the threat pollution poses to the welfare of the American people can be minimized.

EPA's Office of Research and Development conducts this search through a nationwide network of research facilities.

As one of these facilities, the Robert S. Kerr Environmental Research Laboratory is responsible for the management of programs to: (a) investigate the nature, transport, fate, and management of pollutants in ground water; (b) develop and demonstrate methods for treating wastewaters with soil and other natural systems; (c) develop and demonstrate pollution control technologies for irrigation return flows; (d) develop and demonstrate pollution control technologies for animal production wastes; (e) develop and demonstrate technologies to prevent, control or abate pollution from the petroleum refining and petrochemical industries; and (f) develop and demonstrate technologies to manage pollution resulting from combinations of industrial wastewaters or industrial/municipal wastewaters.

This report contributes to that knowledge which is essential in order for EPA to establish and enforce pollution control standards which are reasonable, cost effective, and provide adequate environmental protection for the American public.

William C. Galegar
Director

## ABSTRACT

Analyses of low levels of organic chemicals and microorganisms in subsurface waters and solids are required for realistic assessment of current and potential pollution of ground water, but are particularly difficult to accomplish because of problems in sampling often remote and relatively inaccessible subsurface environments. This report presents procedures currently utilized by the Ground Water Research Branch of the Environmental Protection Agency for sampling for organic pollutants and microorganisms in ground waters and subsurface earth solids.

Technology is described for construction of wells capable of providing representative, uncontaminated samples of ground water in compact alluvial formations at relatively shallow depths and for obtaining cores of subsurface earth solids suitable for organic and microbial analyses in similar circumstances. Methods for acquisition of grab samples of ground water suitable for total organic and microbial analyses and for analyses of volatile organics are presented. Continuous sampling of organics in ground waters lying within approximately 7.5 m (25 ft) of the surface by sampling units utilizing selected absorbents is described, including details of adsorbent columns, configuration of and housings for sampling systems, and sample handling. Procedures for handling and processing of core materials to produce samples amenable to analytical methods for organics and microorganisms are also presented.

The procedures described provide a basic capability for sampling for organic pollutants and microorganisms in relatively shallow subsurface environments, and have potential application in many investigations pertaining to ground-water pollution. Additional research is needed, however, to further evaluate, improve, and extend their capabilities.

This report covers a period from July 1975 to January 1977, and work was completed as of May 1977.

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## ACKNOWLEDGMENT

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# SECTION 1

# INTRODUCTION

The quality of ground water is being increasingly threatened by the continuing and expanding release of both chemical and biological pollutants into the earth's crust as the result of various human activities, particularly those involving disposal of wastes to the land. In order to assess the potential impact of such activities on ground water and, hence, to provide a rational basis for strategies to protect the quality of this valuable resource, the behavior of pollutants in the subsurface and the processes governing this behavior must be elucidated. This requires the capability for detecting and measuring low levels of chemicals and microorganisms in both ground waters and subsurface earth solids. A major problem in development of such analytical capability is the necessity for obtaining uncontaminated, truly representative samples from often remote and relatively inaccessible subsurface environments. Sampling problems are particularly acute in analysis of organic pollutants and microorganisms, which are of major concern because of suspected or known adverse effects on health and the role of microorganisms in processes controlling pollutant behavior and fate. Introduction of contaminants or any loss of sample components in sampling for these parameters is likely to produce an inordinate impact on analytical results because of the low levels usually being determined and the effects of interferences on analytical procedures.

During the course of investigations concerning the movement and fate of pollutants in ground water, the Ground Water Research Branch of EPA has developed and utilized several procedures for sampling organic pollutants and microorganisms in subsurface environments. These procedures are limited in scope, being generally applicable only to ground water and earth solids lying within about 7.5 m (25 ft) of the surface. They represent a first effort, and undoubtedly can and will be greatly improved in future work. However, it is believed that these procedures are sufficiently useful to be of interest to others who may be involved in sampling organic pollutants and microorganisms in the subsurface, either for research or monitoring purposes. Therefore, the details of the methods and equipment currently utilized by the Ground Water Research Branch for sampling organic chemicals and microorganisms in ground water and subsurface earth solids are presented in this report.

## SECTION 2

## CONCLUSIONS

The procedures presented in this report provide a basic capability for sampling for organic pollutants and microorganisms in relatively shallow subsurface environments. The techniques and equipment described herein for well construction and for acquisition of samples from properly constructed wells are satisfactory for sampling ground water for organics and microorganisms at depths down to approximately 7.5 m (25 ft) in compact alluvial formations. The coring and core-processing methods presented may be utilized to sample subsurface earth solids for organics and microorganisms at slightly greater depths in similar geological situations. In their present state of development these procedures have potential application in many situations of possible ground-water pollution, but additional work is needed to further define, improve, and extend their capabilities. In particular, further evaluation of the capabilities of the adsorbents employed for continuous sampling for organics in ground water is needed as a basis for devising optimum sampling systems, and techniques for constructing sampling wells and obtaining uncontaminated cores in situations where the use of drilling fluids appears necessary and for sampling ground water in deeper formations need to be further explored and perfected.

## SECTION 3

## RECOMMENDATIONS

1. The procedures presented in this report, or similar methods based on analogous principles of sample validity and integrity, should be utilized whenever possible in ground-water investigations to sample for organic pollutants and microorganisms in subsurface environments.

2. Additional work should be done to more thoroughly evaluate, improve, and extend the capability of the procedures described herein for sampling for organic pollutants and microorganisms in subsurface environments. In particular, the following needs should be addressed.

   a. The qualitative and quantitative efficiency of various adsorbents for recovering organic compounds from aqueous media should be thoroughly evaluated to provide an improved basis for selection of optimum adsorption systems for sampling organics in ground water.

   b. A non-adsorbing, non-contaminating submersible pump capable of fitting into small-diameter well casings should be developed to permit continuous sampling of organics in ground water at depths in excess of approximately 7.5 m (25 ft).

   c. Technology for constructing wells satisfactory for sampling ground water for organics and microorganisms and for obtaining uncontaminated cores in loose, non-compacted formations, at depths greater than 12-15 m (40-50 ft), and in other situations where use of drilling fluids appears necessary, should be perfected.

## SECTION 4

## SAMPLING OF GROUND WATER

### WELLS FOR SAMPLING

When possible, wells utilized to sample ground water for organic pollutants and microorganisms are drilled and completed using technology which minimizes potential contamination of the aquifer during drilling operations and maximizes the probability that truly representative ground-water samples can be obtained. Existing wells are usually of questionable value for this purpose for a number of reasons, of which the following are principal.

1. Drilling fluids used in ordinary construction of wells for general use are likely to significantly alter the subsurface chemical and microbiological environment in the vicinity of such wells.

2. Water from aquifers other than that of interest may be present in many wells due to their depth, zones of casing, and method of completion.

3. Surface contamination of wells frequently occurs because of poor completion practices.

4. Casing materials and pumping equipment may contribute pollutants to the sampled water or adsorb constituents from it.

Currently, drilling of wells for sampling is accomplished by use of an auger, thus avoiding the use of drilling fluids and the contamination problems associated with the use of these materials. This technique is quite effective for drilling in reasonably compact alluvial materials at relatively shallow depths. Techniques for drilling satisfactory sampling wells at depths greater than 12-15 m (40-50 ft), in very loose formations, and all other situations where the use of drilling fluids appears necessary, remain to be developed.

In the most recent work of the Ground Water Research Branch, 3.8 cm (1.5 in.) I.D. x 4.45 cm (1.75 in.) O.D. Teflon tubing has been used to case that portion of sampling wells extending from a few feet above the water table to the bottom of the borehole. Hence, only this very inert casing material is in contact with ground water in such wells. Because of the high cost of Teflon tubing, 3.8 cm (1.5 in.) I.D. galvanized pipe, coupled to the Teflon with a threaded galvanized coupling, is used to case the upper part of the borehole that lies within the unsaturated zone and is not directly in contact with the ground water.

In some earlier work, 5 cm (2 in.) PVC pipe was utilized for casing of sampling wells. This material is relatively inexpensive and easy to use, but it is less desirable as a casing material than the Teflon tubing-galvanized pipe combination for two principal reasons. First, organic constituents of ground water may be adsorbed on the PVC casing. Second, there is evidence that PVC casing may contribute low levels of organic contaminants to the samples, such as phthalic acid esters used as plasticizers in PVC manufacture and solvents from cements used to join lengths of PVC tubing.

Casing materials are carefully cleaned by washing with soap and water, thorough water rinsing, solvent rinsing, and, finally, rinsing with organic-free water prior to installation in the borehole.

Wells are gravel-packed with clean, washed pea gravel from the bottom of the hole to just below the Teflon-galvanized coupling. The casing is cemented from this point to the surface where a concrete pad approximately 61 x 61 cm (24 x 24 in.) is installed to accommodate sampling equipment and to aid in sealing out surface waters. A screw-type cap on the casing prevents contamination when the well is not in use. The well is completed by thorough pumping, usually with a high-capacity hand diaphragm pump, and is allowed to stabilize for at least 10 days prior to sampling.

## SAMPLING PROCEDURES

### Preliminary Operations

Just prior to actual sampling of ground water, the well from which the samples are to be obtained is thoroughly pumped to insure that all "stale" water standing in the well-bore is removed and replaced by fresh formation water that is truly representative of the water in the surrounding aquifer. This is usually accomplished by means of a "Masterflex" 7015 peristaltic pump powered by a 7545 Variable Speed Drive or 7570 Portable Sampling Drive (Cole-Parmer Instrument Co., Chicago, IL). Ground water is pumped from the water table to the inlet tubing of the pump through "Chemfluor" 6 mm O.D. Teflon tubing, supplied in 366 cm (12 ft) lengths by Chemplast, Inc., Wayne, NJ. The required length of tubing is fabricated by joining appropriate tubing sections with "Taper-Tite" 6 mm I.D. Teflon connector assemblies or 6 mm "Chemfluor" Teflon unions, also supplied by Chemplast, Inc. The tubing is thoroughly cleaned and sterilized prior to use, and is very carefully inserted into the well to minimize introduction of contaminants. Pumping rates in excess of 500 ml/min are ordinarily used, and a volume of water equivalent to at least 10 times the volume of water originally standing in the well casing is removed.

### Acquisition of Grab Samples

Grab samples of ground water for direct microbial analysis, biomass determination by adenosine triphosphate (ATP) analysis, organic carbon determination, and any other desired analyses for which such samples are suitable, are obtained by utilizing the system shown in Figure 1. Ground water is drawn up from the water table through a sterile 6 mm O.D. Teflon tube into a sterile, calibrated 1 liter Erlenmeyer flask by means of a "Masterflex" 7015 peristaltic pump located on the outlet or downstream side of the sampling flask; hence, the sampled water contacts only sterile glass and Teflon during the sampling operation.

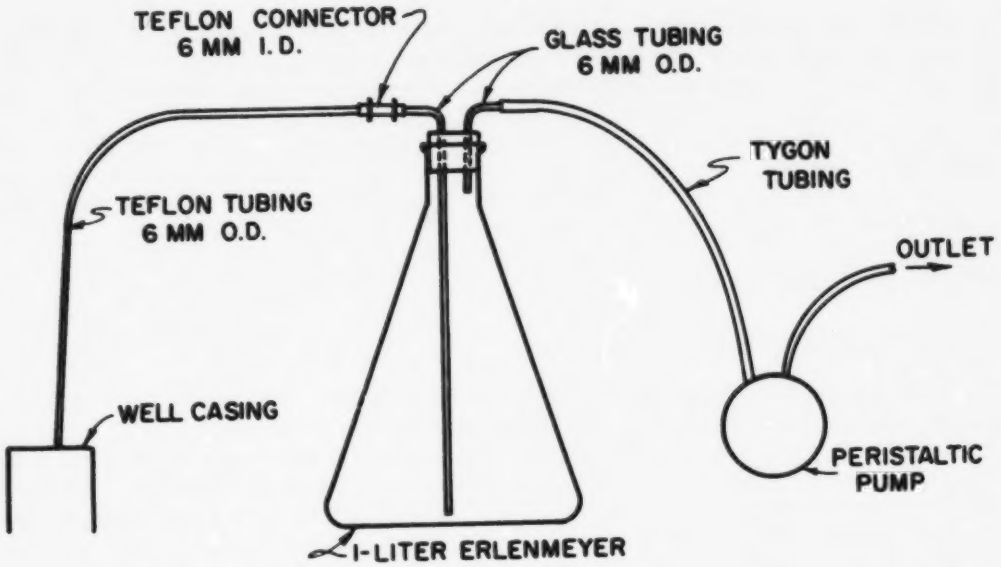


Figure 1. System for acquisition of grab samples of ground water.

Samples for direct microbiological analysis are immediately transferred aseptically to appropriate sample containers, usually 500 ml dilution bottles, using a propane torch for flaming of glassware. Sample bottles are topped off to exclude air, and are packed in ice for shipment to the laboratory for analysis.

Samples to be analyzed for ATP as a measure of total biomass (1) are immediately filtered at the sampling point through 0.45 μ Millipore filters, using sterile Millipore glass filtering equipment with a Gast vacuum pump. The filters are transferred to foil-sealed, sterile 150 ml beakers and immediately frozen on dry ice for return to the laboratory.

Samples for organic carbon analysis are transferred from the sampling flask to thoroughly-cleaned 40 ml vials equipped with Teflon-lined screw caps. They are quick-frozen on dry ice for shipment and storage until analyzed. Care is exercised to insure that adequate space remains in the vials for expansion of the sample upon freezing.

Samples of ground water to be analyzed for highly volatile organics by the Bellar volatile organic analysis (VOA) method (2) are obtained by means of a Teflon bailer. The previously-described system ordinarily used for obtaining grab samples is not suitable for VOA samples because of possible stripping of highly volatile constituents from the sample under the reduced pressures occurring in this system.

The Teflon bailer, shown in Figure 2, is either 46 or 91 cm (18 or 36 in.) in length. It is constructed of 2.5 cm (1 in.) I.D. x 3.8 cm (1½ in.) O.D. Teflon extruded heavy wall tubing plugged at the bottom end with a short length of 2.5 cm O.D. Teflon extruded rod. Water enters the bailer when it is lowered into the well through an 0.8 cm (5/16 in.) hole drilled through the end plug and is prevented from draining out by a 1.9 cm (3/4 in.) diameter glass marble which fits into a conical seat machined into the top of the plug. The plug fits tightly inside of the tube comprising the body of the bailer; hence, no adhesives which might contribute contaminants to sampled water are required to hold it in place. A cable made by weaving together several strands of guage #24 nickel wire is used to raise and lower the bailer.

Bailers are sterilized by autoclaving before use to minimize introduction of contaminants into the wells being sampled. Ground-water samples obtained are very carefully poured from the bailer into clean serum bottles of appropriate size (usually 125 ml), with caution being exercised to avoid turbulence which might result in loss of volatile organics and/or excessive oxygenation of the samples. The serum bottles are topped-off to avoid including gas spaces in the samples and are tightly closed with Teflon-lined septums held in place by aluminum crimp-on seals (Precision Sampling Corporation, Baton Rouge, LA). The sealed VOA samples are packed in ice and returned to the laboratory for analysis at the earliest convenient time.

Continuous Sampling

Sampling of ground water for organic pollutants other than those amenable to the VOA procedure is accomplished by continuous sampling procedures utilizing selected adsorbents to concentrate and recover the organic constituents. These procedures are based on work described by: Junk et al. (3); Breidenbach et al. (4); Buelow, Carswell, and Symons (5); and, Endres and Hörman (6).

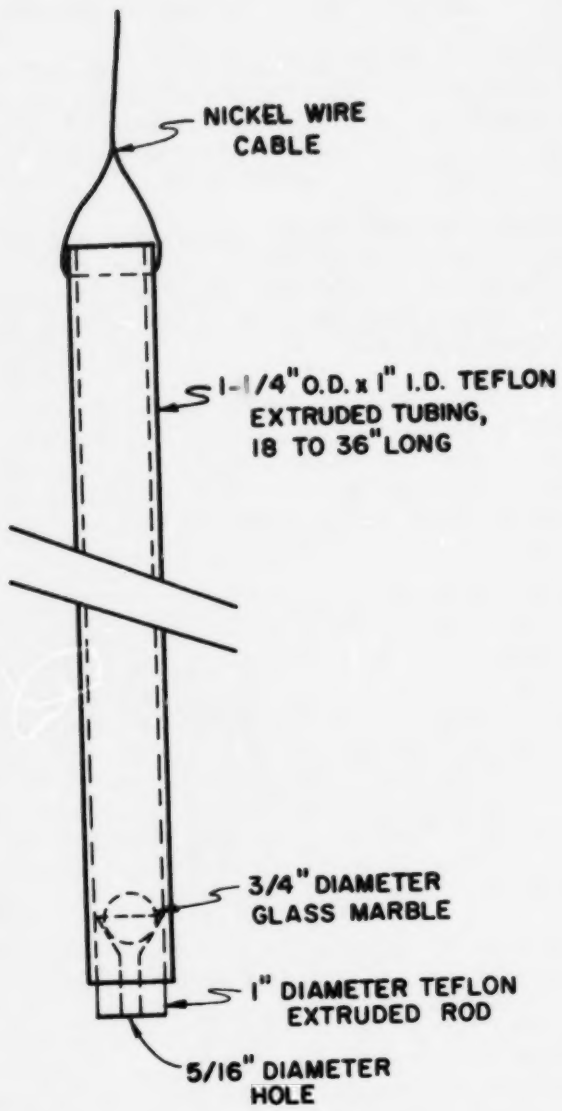


Figure 2. Teflon bailer for ground-water sampling.

A special ground-water sampling system is used in which ground water is pumped directly from the water table through two columns of adsorbent in series by means of a "Masterflex" 7014 peristaltic pump (powered by a 7545-10 Variable Speed Drive) located on the outlet, or downstream, side of the columns (Figure 3). Teflon tubing (6 mm O.D.) conveys the ground water from the zone of saturation to the inlet of the first column. Columns, which vary in configuration depending on the absorbent for which they are designed, are constructed of glass or glass and Teflon. Connecting tubing is 6 mm O.D. glass, and components are joined together by 6 mm I.D. "Taper-Tite" Teflon connectors. These components are thoroughly cleaned to eliminate organic contaminants prior to assembly of the system. Hence, ground water being sampled contacts only clean glass or Teflon (excepting the adsorbents) until it has passed through both adsorbent columns. Loss of solutes by adsorption on sampling equipment and introduction of organic contaminants during the sampling operation are, therefore, virtually precluded.

Sampling systems are installed in specially constructed housings to form self-contained sampling units which are easily transported and set up over wells in the field and which afford protection to the sampling system components against damage by weather, small animals, and accidental breakage. A detailed drawing of a sampling system housing is presented in Figure 4.

Overall dimensions of the housing are approximately 61 cm (24 in.) high, 58 cm (23 in.) wide, and 49 cm (19.5 in.) deep. Access to the interior is gained both through a door in the front side and through the top, which is hinged to open to the rear. A handle on the top facilitates transport of the unit. Space is provided for installation of a "Masterflex" Variable Speed Drive, fitted with as many as three peristaltic pump heads, on the floor at the rear of the housing, and for mounting the control unit for the pump drive on the rear wall. Vent holes in the back and left side walls permit dissipation of heat from the pump drive motor. Two female electrical receptacles are provided inside the housing, mounted in a standard electrical box on the side interior wall. A male motor plug base mounted in the side wall behind this box serves as a receptacle for connecting the housing to an external 110 V electrical source through a suitable extension cord.

Holes of approximately 8 mm (0.3 in.) diameter drilled in removable sections of the housing floor provide passage for the Teflon lines of the sampling systems from the housing into the casing of a well being sampled. The removable floor sections facilitate manipulation of the tubing during installation of a sampling unit over a well and yet permit effective closure of the housing when the unit is in place.

A 12 mm (0.5 in.) aluminum rod, mounted on the side walls of the housing by "Flexaframe" foot plates (Fisher Scientific Co., Pittsburgh, PA), extends across the interior to serve as the support upon which adsorbent columns are installed. Standard laboratory extension clamps and "Flexaframe" 90° connectors are used for column installation. As many as three pairs of columns can be accommodated by a housing; hence, three different sampling systems may be employed simultaneously for sampling ground water from a single well.

A complete ground-water sampling unit, consisting of a housing containing two complete sampling systems, is shown in Figure 5.

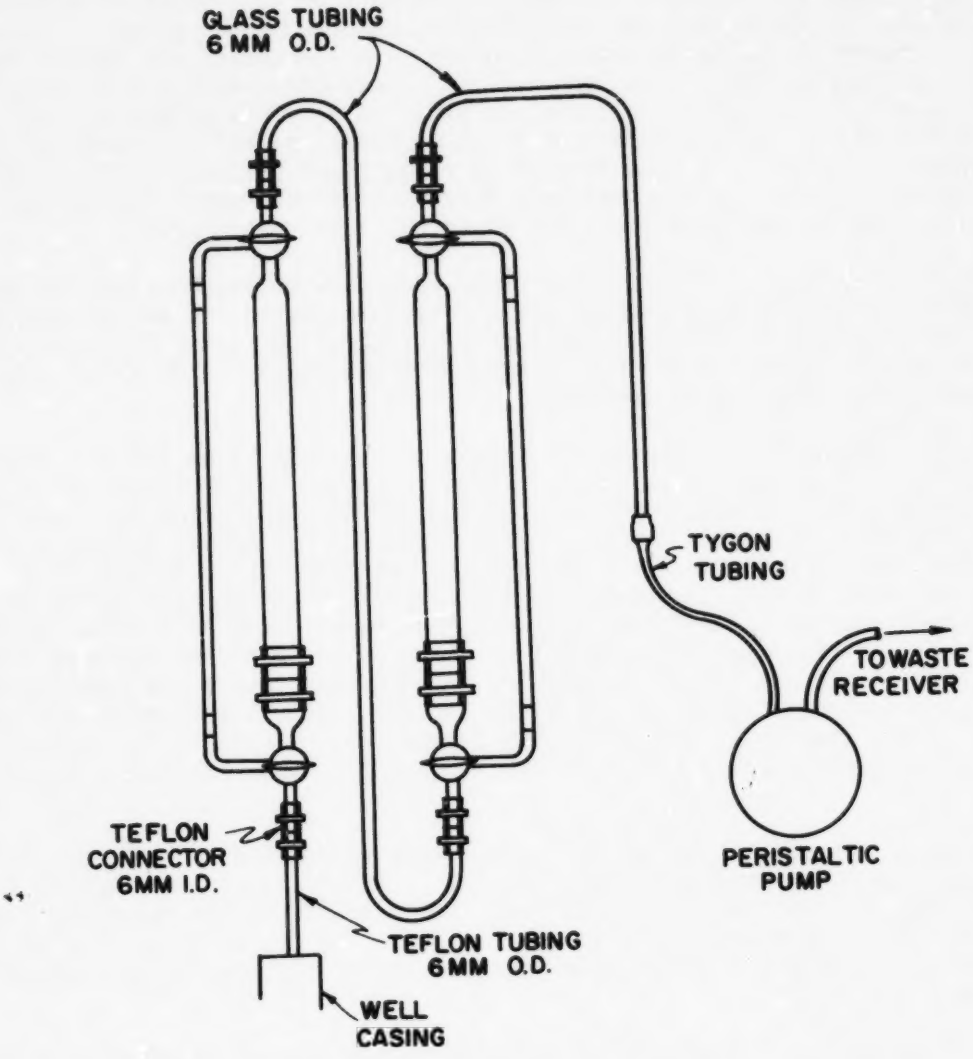


Figure 3. Ground-water sampling system.

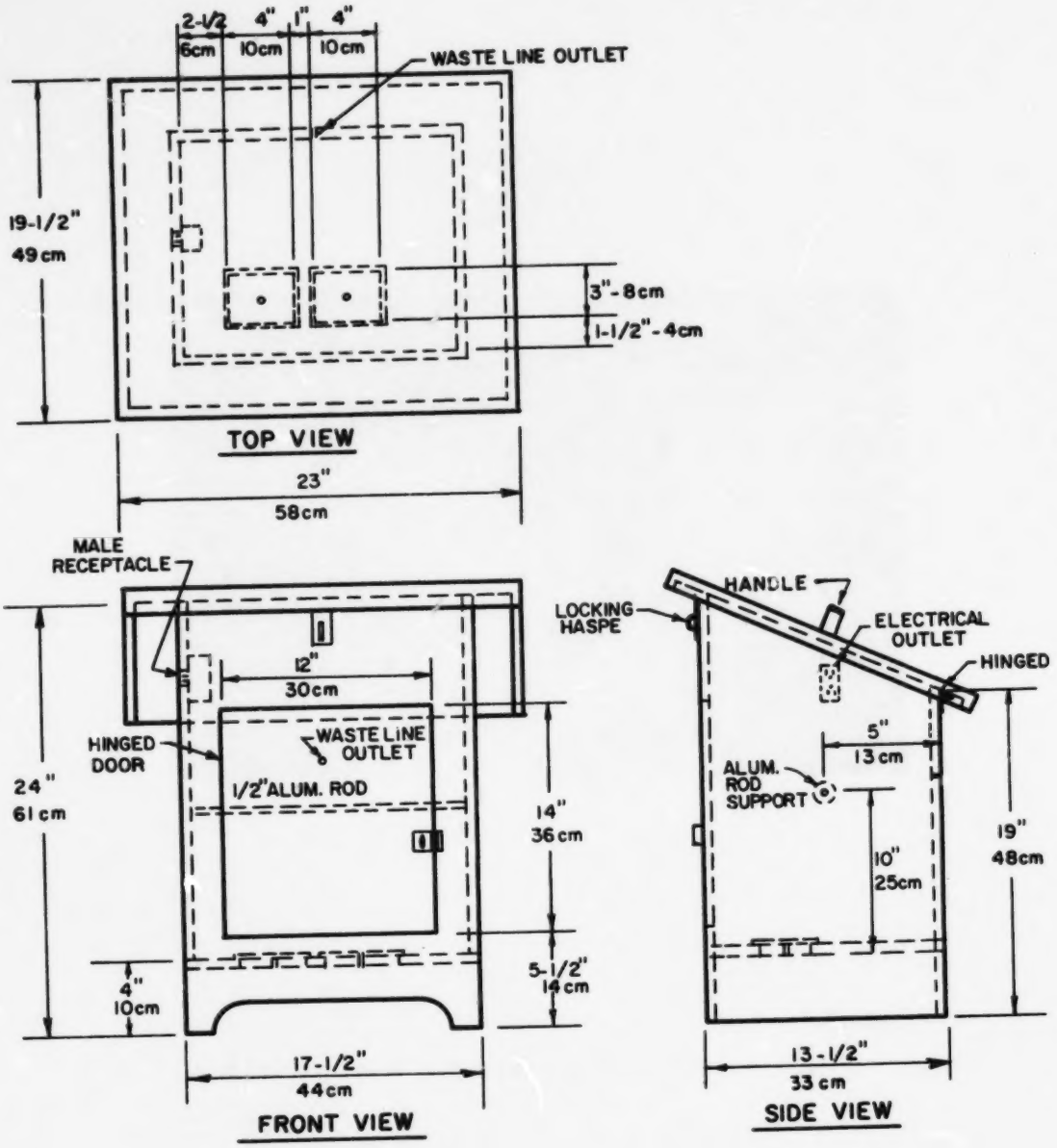


Figure 4. Sampling system housing.

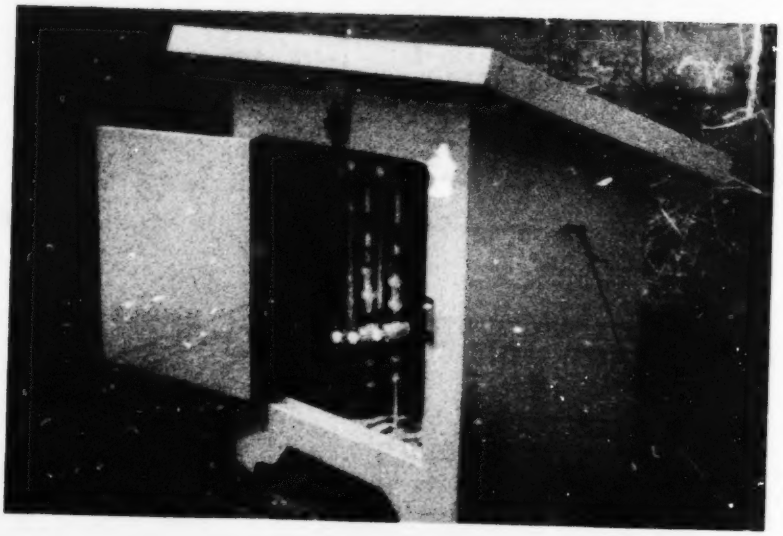

Figure 5. Complete ground-water sampling unit.

Adsorbent columns currently being used are prepared from macroreticular resin (XAD-2, Rohm and Haas Co., Philadelphia, PA), activated carbon (Filtrasorb 200, Calgon Corporation, Pittsburgh, PA), or polyamide (Polyamide Woelm, ICN Pharmaceuticals, Inc., Cleveland, OH).

The XAD-2 resin is purified by sequential solvent extraction with methanol, acetonitrile, and ethyl ether in 2200 ml modified Soxhlet extractors, and stored under methanol until used for column preparation (3). Filtrasorb 200 activated carbon is boiled in a 5% solution of hydrochloric acid in organic-free water for 3 hr, washed thoroughly with organic-free water to remove all chlorides, dried at 150° C for several hours in a clean oven, and stored in glass-stoppered jars until used. Polyamide Woelm (hereafter called PAW) is suspended in distilled water about 2 hr prior to column preparation and washed after packing by passing successively through each column the following solvents: 100 ml water; 100 ml 2-propanol:water (1:1); 200 ml 2-propanol; 100 ml 2-propanol:water (1:1); and, 200 ml water.

XAD-2 and PAW are packed from methanol and water slurries, respectively, to produce 9 x 130 mm adsorbent beds in glass columns fabricated from 12 mm O.D. borosilicate glass tubing. These columns, shown in Figure 6, are equipped with a 3-way Teflon stopcock at each end to permit retention of fluid in the adsorbent during transport and bypassing of the sample stream during sampling. The two column sections are held together by a "Taper-Tite" Teflon connector assembly which permits easy disassembly for packing and elution. The columns are plugged at both ends with solvent-extracted glass wool, with the short, or inlet, sections being completely filled with this material to serve as a filter protecting the adsorbent from particulate matter which might be present in the sample stream. During packing and elution the columns are inverted from the normal sampling position, with the short (sample-inlet) end replaced by a reservoir. After packing and assembly, the XAD and PAW columns are kept sealed and never allowed to become dry.

Carbon adsorption columns are prepared by placing 70 g of dry, purified Filtrasorb 200 in a 30 mm I.D. borosilicate glass column (Figure 7), thus producing a carbon bed approximately 230 mm in length. The columns are equipped with 50/30 ball and socket joints to permit disassembly for packing and removal of the carbon, which is retained in place during sampling by solvent-washed glass wool plugs. Except during actual use, the columns are kept tightly sealed by inserting 6 mm O.D. Teflon plugs into 6 mm I.D. "Taper-Tite" Teflon connectors attached to the column inlets and outlets.

A system comprised of an XAD-2 column and an activated carbon column arranged such that the sampled water first contacts the resin column is currently used for sampling ground water for organic pollutants which are readily amenable to gas chromatography. A system employing two polyamide columns in series is used to sample for pollutants of relatively greater molecular weight and polarity, including particularly those substances capable of forming hydrogen bonds.

For actual sampling, suitable systems are assembled and installed in housings at the laboratory. Organic-free water is pumped through XAD and PAW columns to clear them of solvents, and the systems are sealed by closing appropriate stopcocks and installing "Taper-Tite" connectors containing glass or Teflon plugs on the ends of tubing. The complete sampling units are then transported to the field

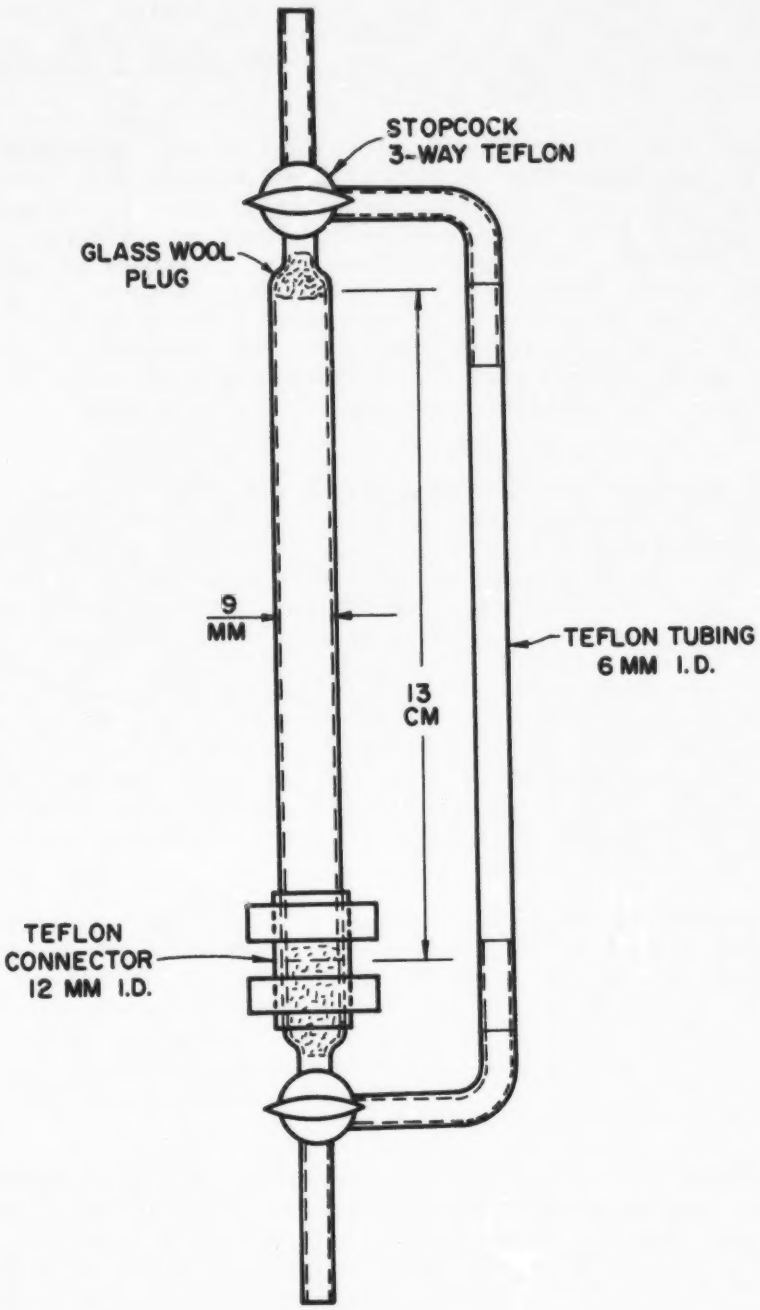


Figure 6. Resin adsorption column.

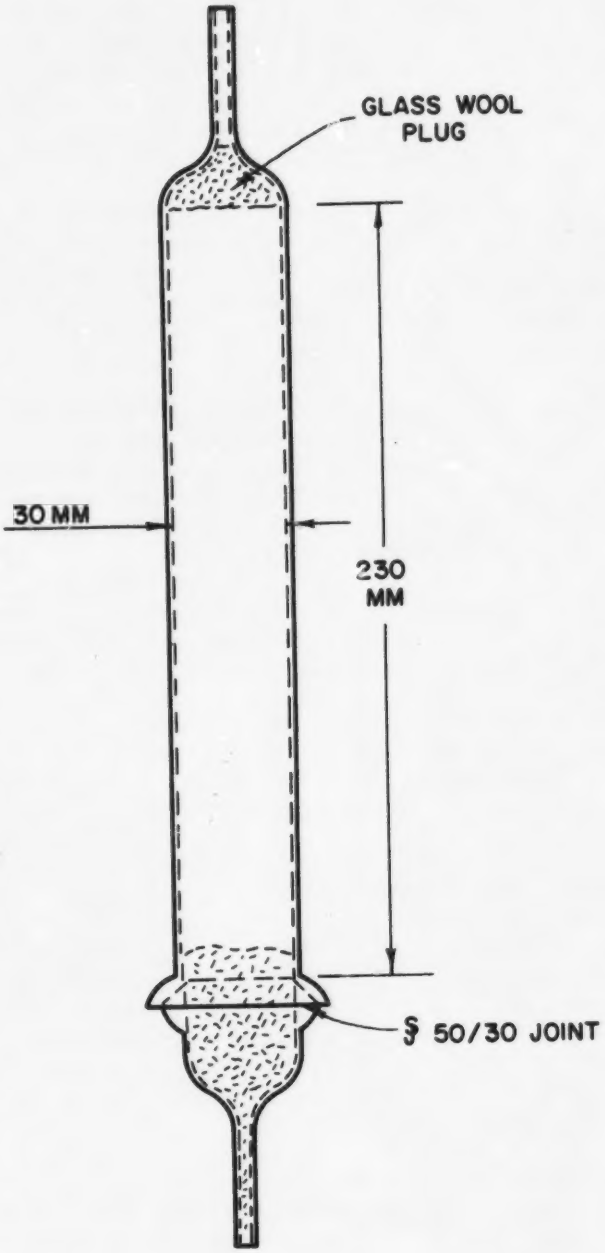


Figure 7. Carbon adsorption column.

and set in place over the casing heads of the wells to be sampled, with the Teflon lines from the sampling systems extending down the well-bores into the ground water. As mentioned previously, the wells are pre-pumped just prior to set-up of sampling units to insure the presence of fresh formation water in the casings when sampling is begun. To preclude the entry of contaminants into the wells, the casing heads are kept tightly covered during the sampling period. This is accomplished by closing them with plastic or metal caps or plugs containing holes just large enough to accommodate the Teflon sampling lines, or by sealing the spaces between the sampling lines and casing walls with heavy aluminum foil.

Sampling is conducted by continuously pumping ground water through the sampling systems for periods approximating seven days at flow rates usually ranging from 10 to 25 ml/min. Flow rates of 15 to 20 ml/min are considered optimum, but are often difficult to maintain because of changing resistances of the columns to fluid flow, principally as a result of accumulation of particulate matter in the glass wool plugs holding the adsorbents in place.

Total volumes of ground water sampled ordinarily range from 100-250 l, and are determined by collecting and measuring the water leaving the sampling systems. For this purpose, water from the outlets of the peristaltic pumps is conveyed by Tygon tubing through the back walls of the sampling system housings into calibrated waste receivers consisting of 32 gal. molded polyethylene trash cans which have been calibrated in liters.

In Figure 8 a sampling unit and waste receivers are shown in place at a field site. As this photograph shows, both unit and receivers are anchored in place by means of wooden stakes and wire cable as a precaution against high winds.

Upon completion of sampling, the adsorbent columns are sealed and the sampling units are immediately returned to the laboratory for disassembly and elution or extraction of the adsorbents. XAD-2 and PAW columns are sealed while completely filled with sample water, while carbon columns are drained of excess liquid before sealing.

For elution, XAD-2 and PAW columns are inverted and the short inlet sections containing glass wool plugs on which considerable particulate matter may have collected are replaced by 300 ml reservoirs. XAD-2 columns are drained of excess water and eluted with 20 ml of acetone followed by 80 ml of chloroform, as suggested by Webb (7). The combined eluates are dried with anhydrous sodium sulfate and reduced to the desired volume in Kuderna-Danish evaporators. PAW columns are eluted with 30 ml of 2-propanol, and the eluates are carefully reduced in volume in rotary evaporators operated at about 100 mm pressure and temperatures not exceeding 25° C.

The contents of activated carbon columns are emptied into clean borosilicate glass pans, dried, and extracted successively with chloroform and ethanol, using essentially the methods of Buelow, Carswell, and Symons (5). The carbon chloroform and carbon alcohol extracts are reduced in volume as needed for further study.

Columns of each adsorbent, which are prepared exactly as those used for sampling but which are allowed to contact only organic-free water, are processed along with the sampling columns to provide blank extracts or eluates.

Figure 8. Sampling unit and receivers installed at field site.

The eluates and extracts produced by the sampling procedures described above comprise essentially uncontaminated concentrates of many of the trace organic constituents of the sampled ground water. They are suitable for analysis by currently available methods to obtain valuable information concerning the identity and quantity of organic pollutants in ground water (8).

## SECTION 5

## SAMPLING OF SUBSURFACE SOLIDS

Subsurface earth solids, as well as ground water, must be sampled and analyzed for organic pollutants and microorganisms if investigations pertaining to the potential impact on ground water quality of activities releasing pollutants into the earth's crust are to be effective. There are several principal reasons for this requirement.

1. Only by analysis of earth solids from the unsaturated zone underlying pollutant-releasing activities can those pollutants which are moving very slowly toward the water table because of sorption and/or physical impediment be detected and their rates of movement and degradation measured. Such pollutants, which probably include a major proportion of organics and microorganisms, are not likely to be detected in ground water until the activities releasing them have been in operation for protracted periods. Because of their potential for long-term pollution of ground water, it is imperative that the behavior of these pollutants in the subsurface be established at the earliest practicable time.

2. Analyses of organic pollutants in solid samples from the zone of saturation are needed for a realistic evaluation of the total extent and probable longevity of organic pollution in an aquifer. Such analyses provide a measure of the quantity of pollutants which are sorbed on aquifer solids and which are in equilibrium with, and in essence serve as a reservoir for, pollutants in solution in the adjacent ground water.

3. Microbial populations which may be involved in the biological alteration of pollutants in subsurface formations are likely to be in such close association with subsurface solids that they will not be present in well waters in numbers which are quantitatively indicative of their presence in the formations; hence, analysis of subsurface solids are needed for accurate evaluation of such populations.

4. Even when the best well construction and ground-water sampling procedures are used, it is difficult to completely eliminate the possibility that contaminating surface microbes may be present in ground-water samples. Solids taken from the interior of cores carefully obtained from the zone of saturation probably provide the most authenic samples of aquifer microorganisms that can be obtained.

Successful sampling of subsurface earth solids for organics and microorganisms requires both acquisition of cores of subsurface solids at desired depths in a manner minimizing potential contamination and proper handling and processing of the core material obtained to insure its integrity and produce samples suitable for determinative analytical procedures.

## ACQUISITION OF CORES

In most of the studies conducted thus far by the Ground Water Research Branch, sampling depths have been shallow enough and strata have been sufficiently compact to permit use of an auger and dry-tube coring procedure for acquisition of cores. In this procedure, a hole is opened to the desired sample depth by augering and a "dry-tube" core sampler consisting of a simple steel tube approximately 46 cm (18 in.) in length and 7.6 cm (3 in.) in diameter is then placed on the bottom of the hole and pushed into the undisturbed formation. The core barrel is usually fitted with a steel drive shoe of slightly smaller inside diameter than that of the barrel to facilitate removal of the core. The core is retained in the barrel by wall friction. The augering-coring procedure is repeated sequentially to obtain a succession of cores until the maximum desired sampling depth is attained. If needed, the borehole opened in the coring operation is completed as a groundwater sampling well.

Contamination problems are minimized in the auger and dry-tube coring procedure, principally because no drilling fluid is used. However, the procedure has been utilized to maximum depths no greater than 25 ft to date and is subject to definite problems when sampling in relatively loose, poorly compacted formations, particularly in saturated zones. The use of a "hollow-stem" auger to hold the hole open during sampling and modification of the sampler to provide better retention of the core material in the tube offer some hope for extending the utility of the procedure, but these techniques have not been evaluated in the field.

For deeper sampling where use of drilling fluids is necessary, limited use has been made of a piston sampler for collecting organic samples. The piston sampler employs a sample tube identical to the dry-tube core barrel but drilling fluid pressure is used as the driving force. The sampler sits on the bottom of the borehole at the end of the drill stem. A shear pin maintains the sample tube in an "up" position until a vent plug is dropped down the drill stem to the sampler. Pressure of the drilling fluid then shears the pin, forcing the tube through the bottom of the hole into the uncored material below the borehole. Considerable additional work is needed, however, to develop optimum methods for obtaining core samples with the piston sampler and to evaluate the efficiency of this device for sampling of organics and microorganisms. In particular, the extent of contamination of cores by drilling fluids and methods for avoiding such contamination need to be further explored.

## HANDLING AND PROCESSING OF CORE MATERIALS

As soon as a core is obtained, the drive shoe is removed and the sample tube is placed into a hydraulic extruding device (Figure 9). As the core sample is forced out of the tube, the first 5 to 8 cm (2-3 in.) are cut off with a sterile scalpel and discarded, or used for analyses of chemical or physical parameters.

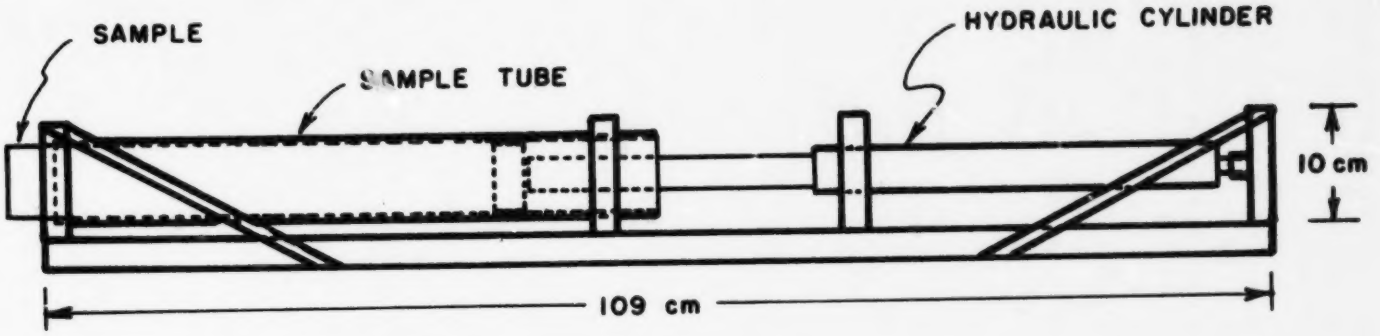


Figure 9. Core extruding device.

The center of the core is then subsampled to obtain sample material suitable for microbial analysis by pushing a sterile 1.3 cm (0.5 in.) I.D. stainless steel tube into the core for about 15 cm (6 in.), as shown in Figure 10. The subsample is extruded with a sterile rod into appropriate containers. By subsampling the interior of the original core, microbial contamination from the core barrel and coring operations can be prevented (9).

The type of microbiological sample container which is used is dependent on the type of analysis to be performed. For culturing of aerobic organisms any sterile container is suitable if analyses are to be performed within a few hours. If there is to be a significant delay before the sample is used, care is exercised to keep the sample in a manner that prevents major changes in the microbial content. Thus, polyethylene bags, which allow the passage of air but not water vapor, are used as sample containers if aerobic organisms are to be cultured in order to give the samples access to air and yet keep them from drying. Samples are maintained if possible at the temperatures at which they are sampled. Otherwise they are refrigerated.

Since subsurface environments of any depth are usually reducing in nature, the enumeration and identification of anaerobic microorganisms is essential if the total microbial composition of the system is to be known. Because many anaerobic bacteria are known to be extremely sensitive to oxygen, it is important that samples which will be used in anaerobic culturing procedures be handled in a manner that minimizes exposure to air. This is accomplished by extruding subsamples into sterile glass tubes from which the air is replaced quickly with an oxygen-free gas. Two methods have been utilized for air removal and replacement. In one method the sample tube is closed with a cotton plug and placed in an anaerobic jar from which the oxygen is removed either by catalytic means or by the use of a vacuum pump-replacement gas system (usually oxygen-free nitrogen). In the second method, the sterile glass tube containing the subsample is fitted with a gas-tight rubber septum stopper. A needle is pushed through the septum and the tube is evacuated with a vacuum pump and filled with a sterile, oxygen-free gas such as nitrogen. This process of evacuation and gas replacement is repeated at least three times.

Samples to be analyzed for ATP (Adenosine Triphosphate) content are taken in the same manner as microbial samples. Subsamples are placed into sterile metal cans which are closed and immediately placed into liquid nitrogen contained in an insulated polystyrene box. Frozen samples are returned to the laboratory on dry ice and stored at -45° C in a low temperature freezer until being analyzed.

After a sub-core for microbial analysis has been removed from the parent core, a 10 cm (4 in.) length of core material for organic analysis is obtained. This is achieved in two steps. First, a 5 cm (2 in.) section of core is extruded from the barrel, carefully detached by means of a clean spatula or scalpel, and dropped directly into a thoroughly cleaned 14 x 8 x 5 cm (5-11/16 x 3-1/4 x 2 in.) disposable aluminum baking pan. The procedure is then repeated to obtain a second 5 cm section of core in the same pan. The pan is covered tightly with clean aluminum foil and placed in an insulated polystyrene box containing liquid nitrogen to quick-freeze the sample material. A maximum of two organic samples are usually obtained from a single 46 cm parent core. Organic and microbial samples are ordinarily taken from the same location in the core, as shown in Figure 11, to facilitate data correlation.

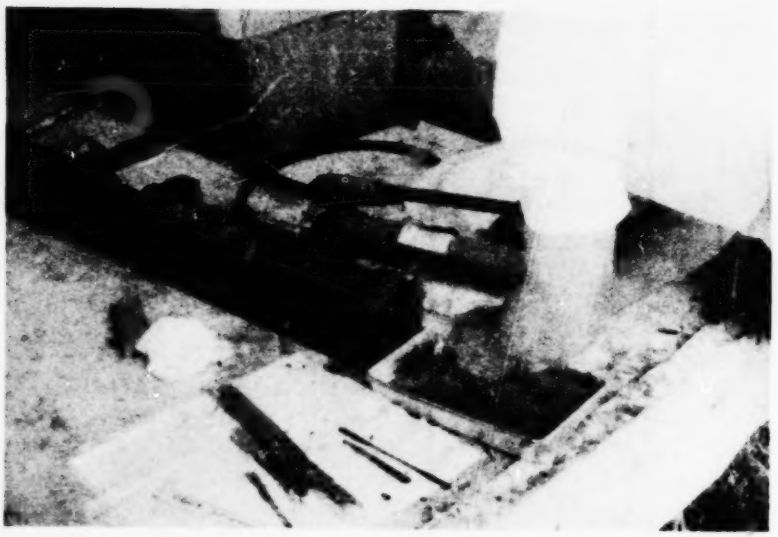

Figure 10. Obtaining a subsample for microbial analysis from a parent core.

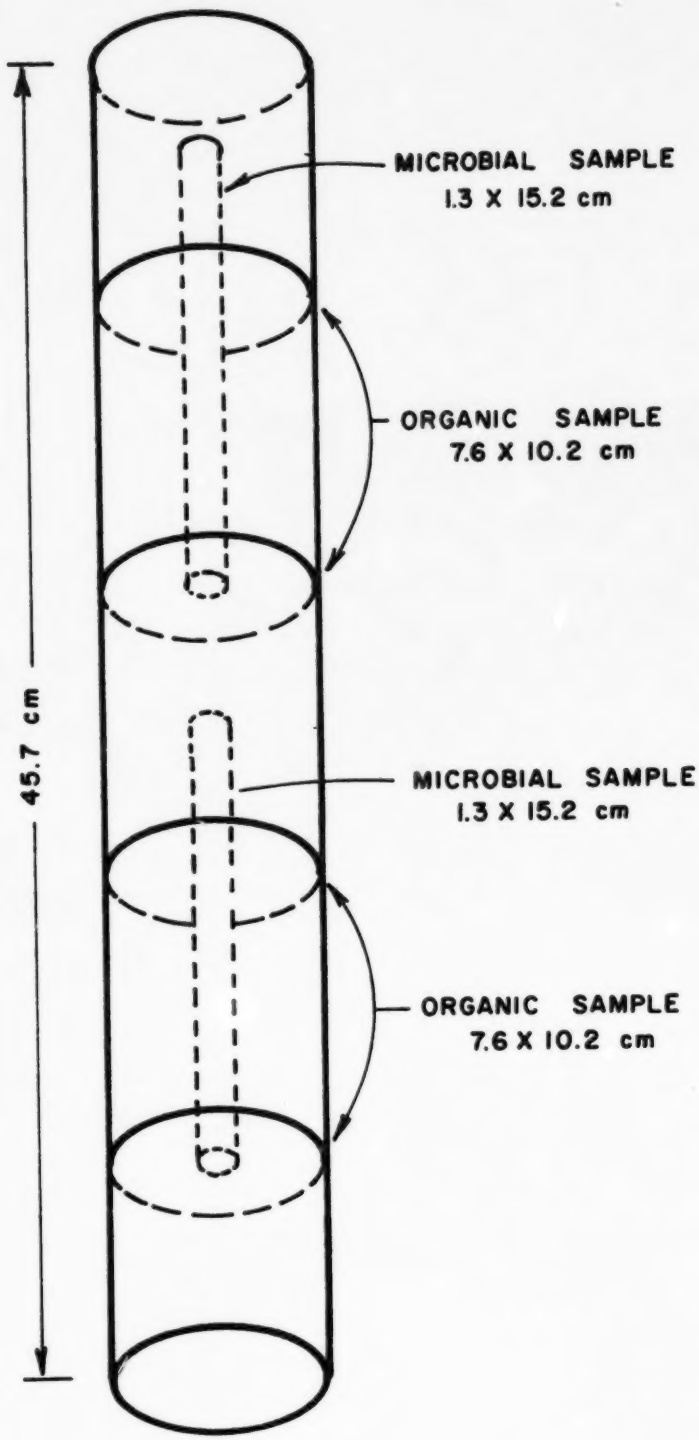


Figure 11. Typical location of microbial and organic samples in a parent core.

The frozen samples of earth solids are returned to the laboratory on dry ice, stored temporarily at -45° C in a low-temperature freezer, and freeze-dried as soon as possible in a bulk type freeze drier (Universal Sub-Mobile 15, The Virtis Company, Inc., Gardiner, NY). Each sample of dried solids is carefully crushed and mixed to obtain a better degree of homogeneity. These samples are then transferred to thoroughly cleaned 475 ml (16 oz) wide-mouth jars with Teflon lined caps and stored at -45° C until subjected to further processing or analysis.

Samples of dried core material are subjected to gross organic analysis, such as total organic carbon, without further processing. Samples suitable for more definitive organic analysis, including identification of individual compounds, are prepared by solvent extraction of the solid samples. This is achieved by extracting samples consisting of approximately 150 g of dried solids in large Soxhlet extractors for 48 hr with an azeotropic mixture of 87% chloroform and 13% methanol. Teflon fiber extraction thimbles (43 x 123 mm "Zitex," Chemplast, Inc., Wayne, NJ) are used. New thimbles are pre-extracted for 72 hr with chloroform-methanol (87:13) to remove manufacturing impurities, while used thimbles are washed in detergent solution in an ultrasonic cleaner, thoroughly rinsed in tap and distilled water, and extracted overnight with chloroform-methanol prior to reuse.

Chloroform-methanol extracts are passed through pre-extracted glass fiber filters into 500 ml round bottom flasks, using Millipore 47 mm glass filter holders modified with 24/40 glass joints on the outlets to eliminate possible contamination from use of rubber stoppers. The filtered extracts are then reduced in volume by means of rotary evaporators to provide samples suitable for comparison and identification studies.

## SECTION 6

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